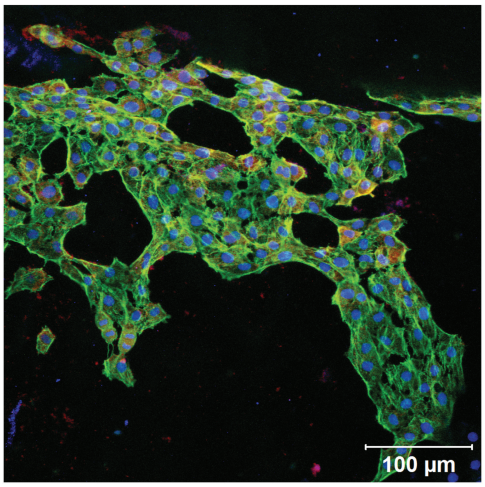
Highlights from Microscopy AND Microanalysis

Biological Applications

Design and Fabrication of a Three-Dimensional *in vitro* System for Modeling Vascular Stenosis by RS Jones, PH Chang, T Perahia, KA Harmon, L Junor, MJ Yost, D Fan, JF Eberth, and RL Goodwin, *Microsc Microanl* 23(4) (2017) 859–71

Vascular stenosis triggers adaptive cellular responses that induce adverse remodeling, which can progress to partial or complete vessel occlusion. Despite its severity, cellular interactions and biophysical cues that regulate pathological progression are poorly understood. We report the design and fabrication of a three-dimensional *in vitro* system to model vascular stenosis so that specific cellular interactions and responses to hemodynamic stimuli can be investigated. Tubular cellularized constructs (cytotubes) were produced using a collagen casting system to generate a stenotic arterial model. Fabrication methods were developed to create cytotubes containing co-cultured vascular cells, where cell viability, distribution, morphology, and contraction were examined (Figure). Fibroblasts, bone marrow primary cells, smooth muscle cells (SMCs), and endothelial cells (ECs) remained viable during culture and developed location- and time-dependent morphologies. We found cytotube contraction to depend on cellular composition, where SMC-EC co-cultures adopted intermediate contractile phenotypes between SMC- and EC-only cytotubes. Our fabrication approach and resulting artery model can serve as an *in vitro* 3D culture system to investigate vascular pathogenesis.

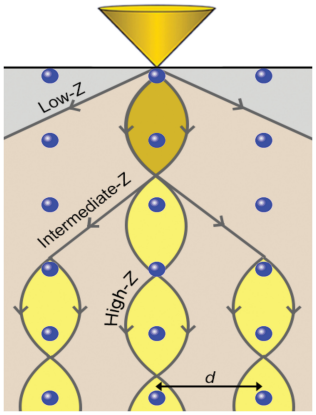


ECs were seeded onto luminal surfaces of cytotubes. After five days of culture, extensive cortical arrangements of f-actin (green staining) associated with adjacent ECs along the luminal boundary indicated the potential development of tight junctions between these cells. Some ECs had smooth muscle actin filaments (red staining), although most exhibited increasing confluency and squamous morphology. Cell nuclei are shown with blue staining (DAPI).

Techniques and Material Applications

Simplifying Electron Beam Channeling in Scanning Transmission Electron Microscopy (STEM) by RJ Wu, A Mittal, ML Odlyzko, and KA Mkhoyan, *Microsc Microanal* 23(4) (2017) 794–808.

Sub-angstrom scanning transmission electron microscopy (STEM) allows quantitative column-by-column analysis of crystalline specimens by measuring the intensity of scattered electrons at a particular location. However, electron beam channeling causes oscillations in the STEM probe intensity as the beam propagates through a specimen, which affects the scattered beam intensity. Understanding the parameters that control this complex behavior is critical for interpreting experimental STEM results. Herein, theoretical analysis reveals that intensity oscillations during specimen propagation are partially regulated by changes in the beam's angular distribution. Three distinct sample-based regimes of channeling are observed: the high-atomic-number (Z) regime, in which strong atomic scattering leads to significant angular redistribution of the beam; low-Z regime, in which the probe's initial angular distribution controls oscillations; and intermediate-Z regime, in which the behavior is mixed. These contrasting regimes are shown to exist for various beam parameters. These results provide a new understanding of the occurrence and consequences of channeling phenomena and the conditions under which the effect is influenced by characteristics of the electron beam and sample.



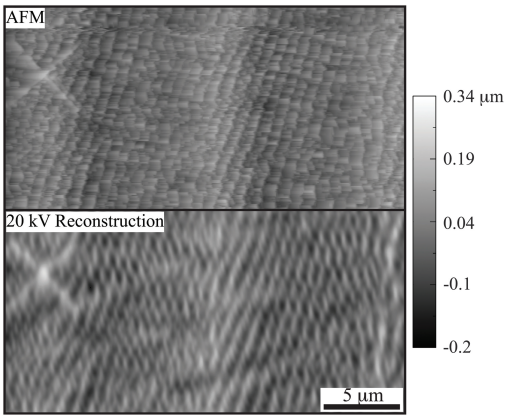
Schematic diagram showing the paths of the electron beam intensity from the STEM probe as it propagates in low-, intermediate-, and high-Z crystals. Blue spheres represent atomic positions within a crystal where *d* is the inter-column spacing.

Microscopy AND Microanalysis

Techniques and Materials Applications

Reconstruction of Laser-Induced Surface Topography from Electron Backscatter Diffraction Patterns by PG Callahan, MP Echlin, TM Pollock, and M DeGraef, *Microsc Microanl* 23(4) (2017) 730–40

Various techniques are available for measuring surface topography, such as atomic force microscopy (AFM) and interferometry. We demonstrate that sample surfaces also can be reconstructed from electron backscatter diffraction (EBSD) patterns collected using a commercial EBSD system. The surface topography is reconstructed from the surface normals at all points in an EBSD scan using a Fourier space technique. Monte Carlo simulations of electron scattering in the sample are used to determine the deviation from specular reflection as a function of surface inclination. This deviation is used as a correction in determining the surface normal at each point from the location of the maximum background intensity in the collected EBSD patterns. Here the laser-induced periodic surface structures (LIPSS) of a femtosecond laser-ablated nickel sample were reconstructed from EBSD scans collected at 5 kV and 20 kV and were in agreement with measurements made with AFM (figure). The technique requires no modification of commercial EBSD systems and enables observation of surface topography evolution during *in situ* experiments, for example during plastic deformation or phase transformations.



Height maps of the same region on the surface of a femtosecond laser-ablated nickel sample from an AFM scan and a reconstruction from EBSD patterns collected at an accelerating voltage of 20 kV. The x-shaped fiducial marker visible in both imaging modalities is deposited platinum used to locate the same region.